

Association between 25(OH)-vitamin D and testosterone levels: Evidence from men with chronic spinal cord injury

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Objective: As an independent linear association between 25-hydroxyvitamin D (25(OH)D) and testosterone levels is controversial, this study aimed to explore this topic in men with chronic spinal cord injury (SCI), who exhibit a high prevalence of both androgen and vitamin D deficiency.

Design: Forty-nine men with chronic SCI consecutively admitted to a rehabilitation program underwent clinical/biochemical evaluations.

Results: Deficiency of 25(OH)D (<20 ng/mL) was found in 36 patients (73.5%). They exhibited significantly lower total testosterone and free testosterone levels, higher parathyroid hormone (PTH) and HOMA-IR, a poorer functional independence degree, and were engaged in poorer weekly leisure time physical activity (LTPA). Significant correlates of 25(OH)D levels were: total testosterone, free testosterone, PTH, functional independence degree and weekly LTPA. At the linear regression models, lower 25(OH)D levels were associated with both lower total and free testosterone after adjustment for age, smoking, alcohol consumption, comorbidities and HOMA-IR. However, after full adjustment, also including functional independence degree, BMI and LTPA, only the association of lower 25(OH)D with lower free testosterone was still significant.

Conclusion: In men with SCI, 25(OH)D correlates with total and free testosterone and exhibits an independent linear association with free testosterone. Regardless of this independent link, hypovitaminosis D and androgen deficiency are markers of poor health, sharing common risk factors to take into account in the rehabilitative approach to patients with SCI.

Keywords: Androgen deficiency, Hypogonadism, Hypovitaminosis D, Paraplegia, Tetraplegia

Introduction

Vitamin D, beside its well known role in bone health and calcium metabolism,¹ extends its actions well beyond calcium homeostasis. An increasing body of observational data has linked low serum levels of 25-hydroxyvitamin D (25(OH)D) to an increased risk of a variety of chronic diseases, including cancer,² diabetes,^{3,4} hypertension,⁵ autoimmune and musculoskeletal diseases,⁶ depression⁷ and cardiovascular diseases.^{6,8} Biological actions of vitamin D are mediated by the vitamin D receptor (VDR), which is almost

ubiquitously expressed in human cells, making biologically plausible a wide spectrum of pleiotropic effects of vitamin D.

The expression of VDR and vitamin D metabolizing enzymes in the male reproductive tract has suggested a biological link between vitamin D and male reproductive hormones.⁹ An independent association between 25(OH)D and total testosterone levels has been reported in two studies.^{10,11} However, in the European Male Aging Study (EMAS), a large cross-sectional study among 3369 community-dwelling men, the linear association was lost after adjusting for health and lifestyle factors, although a weak association persisted between vitamin D deficiency and both secondary and compensated primary hypogonadism.¹²

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Actually, low vitamin D and androgen deficiency might represent markers of a poor health status, sharing common underlying aetiologies. If an independent association between vitamin D deficiency and low testosterone really exists, it should be demonstrable, regardless of confounders, in different populations, including men who exhibit a high prevalence of risk factors for both hypovitaminosis D and biochemical androgen deficiency. In this view, men with spinal cord injury (SCI) could represent a suitable clinical model of study.

A higher prevalence of vitamin D deficiency has been reported in patients with SCI than in able-bodied population.¹³ In these subjects a combination of poor sunlight exposure, inadequate diet, comorbidities and obesity, highly prevalent after SCI, along with the intake of medications that induce hepatic microsomal enzymes, accelerating vitamin D metabolism, could be involved in the pathogenesis of the hypovitaminosis D.¹⁴ A decline in serum testosterone levels in men after SCI has been also reported in the last decade.^{15–20} The very high prevalence of biochemical testosterone deficiency (up to 83%) in men with acute SCI¹⁵ is not surprising, considering the effects of acute physical distress and systemic illness on testosterone levels. Nevertheless, higher rates of low serum testosterone have been also reported in men with chronic SCI.^{16–20} Although the pathophysiology of the high prevalence of biochemical androgen deficiency in men with chronic SCI has not yet been clarified, we recently demonstrated that modifiable life-style related risk factors, such as poor leisure time physical activity (LTPA) and high body mass index (BMI), could represent strong independent predictors of low testosterone in these subjects.¹⁹ Interestingly, poor physical activity and overweight could also contribute to the hypovitaminosis D: not only physical inactivity favours a poor sunlight exposure, but also it results in low energy expenditure, thus leading to adiposity excess, which in turn decreases bioavailability of vitamin D.²¹ However, in a recent report,²² no correlation was found between 25(OH)D and testosterone levels in 29 men with chronic SCI, while a significant correlation was found in 17 age matched, able-bodied controls.

In this study we analyzed the relationship between 25(OH)D and testosterone levels in a larger series of men with chronic SCI, as an association is expected in this population, that exhibits a high prevalence of both hypovitaminosis D and biochemical androgen deficiency, as well as a wide spectrum of risk factors for both of these conditions.

Methods

Study population

Forty-nine male patients, aged 47.5 ± 17.3 years, consecutively admitted to a rehabilitation program at the San Raffaele Institute of Sulmona because of traumatic SCI, were included in the study. All patients had a documented history of neurologically stable SCI for more than 1 year. No patient received testosterone or vitamin D replacement therapy. Sixteen patients took gabapentin for neuropathic pain relief. Coexisting chronic illness were registered: they included heart diseases, hypertension, diabetes and depression. No patient had acute illness hindering the rehabilitative program. All participants were requested to sign a written informed consent and the study was approved by the local ethics committee.

Clinical examination

Patients underwent detailed neurological examination according to the guidelines of the International Standards for Neurological Examination and Functional Classification of Spinal Cord Injury²³ and the American Spinal Injury Association (ASIA) protocol was used to define both level and completeness of the lesion.²³ According to the ASIA impairment scale, patients with complete lesion and no sensory or motor function preserved in the lowest sacral segment were categorized as A, whereas patients with incomplete lesion were categorized as B–D. Category B indicated sensory incomplete lesion (including segments S4–S5); category C indicated sensory and motor incomplete lesion where more than half of the 10 pairs of key muscles have strength of less than 3 on a scale of 0–5; category D indicated sensory and motor incomplete lesion with at least half of the key muscles having strength greater than or equal to 3.

Functional independence degree was assessed at admission by the Barthel Index. It is a 10-item instrument measuring disability in terms of the level of functional independence obtainable by a patient in personal activities of daily living (ADL), giving a final score that ranges from 0 (totally dependent) to 100 (totally independent).²⁴ Because of its reliability and validity, the Barthel index represents a standard measure of disability in different categories of patients, including spinal cord-injured subjects.²⁵

Body weight was taken with patients wearing light clothing, using a professional mechanical chair scale (Wunder SA BI Srl, Monza, Italy). After placing the patient in a bed, his legs were straightened, his head was positioned in the Frankfurt plane, and his feet were placed in dorsal flexion. Height was determined

by an elastic tape, measuring segmentally the heel to knee, the knee to hip and the hip to head distances. The body mass index (BMI) was calculated in kilograms per square meter (kg/m^2).

Assessment of Leisure Time Physical Activity

Leisure Time Physical Activity (LTPA) includes physical exertion-related activities, that people choose to do in their free time, and in case of patients with SCI it includes walking or wheeling and certain sports played in a gym. LTPA was quantified using the LTPA Questionnaire for people with Spinal Cord Injury (LTPAQ-SCI).²⁶ This is a SCI-specific measure of minutes of mild, moderate, and heavy intensity LTPA performed over the previous 7 days, according to the physical activity guidelines for people with SCI.²⁷ The questionnaire takes less than 5 minutes to complete and can be self-administered. Participants used an intensity classification chart to distinguish between mild, moderate, and heavy intensity LTPA, based on perceived psychophysical effort. For each intensity level, participants recalled the number of days, over the past 7 days, that they performed LTPA at each intensity. Next, they recalled how many minutes/day they usually spent doing LTPA at that intensity. The scale was scored by calculating the total number of weekly minutes of activity performed at each intensity (number of days of activity \times number of minutes of activity). Total weekly minutes were divided by 60 to obtain the number of hours of activity performed over the past week. Due to the high correlation of total LTPAQ-SCI scores with mild, moderate and heavy sub-scores,¹⁹ only total LTPAQ-SCI scores were used for analyses

Hormones, biochemistry and haematology

A single fasting morning venous blood sample was obtained from each subject between 8:00 and 9:00 A.M. Serum 25(OH)D levels were determined using a chemiluminescent immunoassay (LIAISON[®], DiaSorin, Saluggia, VC, Italy) with intra- and inter-assay coefficients of variation (CV) of 4.5% and 8.5%, respectively. 25(OH)D levels were classified into sufficient ($\geq 30 \text{ ng/mL}$), sub-optimal (20–29.9 ng/mL) and deficient ($< 20 \text{ ng/mL}$), according to previously recommended cut-off points;²⁸ in particular, values $< 20 \text{ ng/mL}$ (or $< 50 \text{ nmol/L}$) were considered inadequate or not sufficient.²⁹ Serum levels of total testosterone were measured by chemiluminescence immunoassay, using kits from Ortho-Clinical Diagnostics (Johnson & Johnson, New Brunswick, NJ, USA). The lower detection limit for testosterone quantitation was

0.03 nmol/L ; the within- and between-assay CV of testosterone measurements were 2.5% and 4.9%, respectively. A total testosterone level below 300 ng/dL ($< 10.4 \text{ nmol/L}$) indicated a biochemical androgen deficiency, according to the Endocrine Society guidelines.³⁰ Sex hormone binding globulin (SHBG), luteinizing hormone (LH), parathyroid hormone (PTH) and insulin levels were assessed by chemiluminescence immunoassay, using kits from Medical Systems (Genova, Italy). Free testosterone levels were derived from total hormone, SHBG, and albumin concentrations as previously described,³¹ using a web-based calculator (<http://www.issam.ch/freetesto.htm>). All the other biochemical/haematological measurements were performed using standard methods and commercial kits (Instrumentation Laboratory Company, Lexington, MA, USA). Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR), according to the formula: insulin (mU/L) \times glucose (m/dL)/405.³²

Statistical analysis

Statistical analysis was performed using the R statistical software (version 2.15.2, 2012, The R Foundation for Statistical Computing, Vienna, Austria). After assessing the distribution of data with Shapiro–Wilk test, Wilcoxon rank-sum test and unpaired two-sided Student's *t* test were used, as appropriate, for data analysis. Proportional differences were assessed by the χ^2 test or the Fisher exact test as appropriate. Correlations were evaluated using the Spearman's or Pearson's correlation test as appropriate. The association of 25(OH)D levels with total and free testosterone levels was evaluated using separate linear regressions analyses of log-transformed values. Adjustments were made for age, smoking, alcohol consumption, coexisting illness, HOMA-IR, functional independence degree, BMI and weekly LTPA. Multicollinearity was assessed using the variance inflation factors (VIFs).

Results

Cohort characteristics and 25(OH)D correlates

A deficiency of 25(OH)D ($< 20 \text{ ng/mL}$) was observed in 36 patients (73.5% of the study population): in this group 25(OH)D ranged from 4.12 to 19.1 ng/mL . Eleven men (22.4% of the study population) exhibited sub-optimal 25(OH)D levels, ranging from 20.5 to 24.9 ng/mL . Only two men exhibited 25(OH)D levels $> 30 \text{ ng/mL}$.

Table 1 shows the characteristics of the study population according to 25(OH)D status. Patients with 25(OH)D deficiency exhibited significantly lower total

Table 1 Characteristics of the study population categorized by vitamin D status

Characteristic	25(OH)D (ng/mL)		P value
	<20 (n = 36)	≥20 (n = 13)	
Age (yr)	47.0 [20.0–78.0]	49.0 [33.0–76.0]	0.7
Current smokers–no. (%)	10 (27.8)	8 (61.5)	0.06*
Alcohol intake ≥1 day/week–no. (%)	25 (69.4)	11 (84.6)	0.5*
Body mass index (Kg/m ²)	24.8 ± 4.8	23.6 ± 4.2	0.4
25(OH)D (ng/mL)	11.8 ± 4.2	24.4 ± 4.0	<0.0001
Total testosterone (ng/dL)	328.0 ± 170.0	496.0 ± 132.0	0.002
Free testosterone (pg/mL)	87.1 ± 42.8	151.2 ± 46.1	<0.0001
LH (U/L)	4.3 [0.7–23.8]	3.6 [1.1–7.8]	0.4
SHBG (nmol/L)	18.1 [2.8–64.0]	12.5 [5.7–26.3]	0.4
PTH (pg/mL)	35.1 [7.5–116.6]	18.7 [6.0–41.2]	0.002
Calcium (mg/dL)	9.4 ± 0.5	9.5 ± 0.3	0.4
Phosphorus (mg/dL)	3.9 ± 0.6	3.9 ± 0.6	0.8
Glucose (mg/dL)	88.0 [56.0–145.0]	86.0 [72.0–101.0]	0.9
Insulin (mU/L)	8.5 [1.9–30.7]	5.3 [3.0–9.6]	0.007
HOMA-IR	2.0 [0.3–8.1]	1.0 [0.6–2.1]	0.004
LDL-c (mg/dL)	101.2 [53.6–205.6]	108.6 [68.4–153.2]	0.5
HDL-c (mg/dL)	38.0 ± 9.8	42.8 ± 6.5	0.08
Triglycerides (mg/dL)	122.0 [42.0–358.0]	107.0 [52.0–276.0]	0.03
AST (U/L)	17.0 [8.0–69.0]	16.0 [9.0–28.0]	0.9
ALT (U/L)	17.0 [5.0–78.0]	16.0 [9.0–33.0]	0.7
γ-GT (U/L)	24.5 [13.0–55.0]	23.0 [12.0–80.0]	0.3
Level of lesion–no. (%)			
Cervical spine	19 (52.8)	3 (23.0)	0.1 [#]
Thoracolumbar spine	17 (47.2)	10 (77.0)	0.1*
Lesion completeness–no. (%)			
ASIA score A (complete)	17 (47.0)	8 (61.5)	0.6*
ASIA score B-D (incomplete)	19 (53.0)	5 (38.5)	0.6*
Barthel index (functional independence degree)	43.0 [3.0–88.0]	58.0 [12.0–83.0]	0.03
LTPA (hours/week)	7.3 ± 4.4	12.3 ± 6.0	0.003
Coexisting illness–no. (%)	24 (66.7)	7 (53.8)	0.6*
CRP>5 mg/L–no. (%)	12 (33.3)	1 (7.7)	0.1 [#]
Gabapentin intake–no. (%)	11 (30.5)	5 (38.5)	0.8*

Data were expressed as mean ± standard deviation when normally distributed, as median [min-max] for parameters with non-normal distribution: unpaired two-sided Student's *t* test and Wilcoxon rank-sum test were applied to detect differences, according to normal or non-normal distribution of continuous variables. Data were expressed as percentages when categorical: proportional differences were assessed by the χ^2 test (*) or the Fisher exact test ([#]) as appropriate.

25(OH)D = 25-hydroxyvitamin D; LH = Luteinizing hormone; SHBG = sex hormone binding globulin; PTH = parathyroid hormone; HOMA-IR = homeostatic model assessment of insulin resistance; LDL-c = low-density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; γ-GT, gamma-glutamyl transpeptidase; ASIA = american spinal injury association; LTPA = leisure time physical activity; CRP = C-reactive protein. Coexisting illness included: heart diseases, hypertension, diabetes and depression.

To convert the values for 25(OH)D to nmol/L, multiply by 2.5; to convert the values for total testosterone to nmol/L, multiply by 0.03467; to convert the values for free testosterone to pmol/L, multiply by 3.467.

and free testosterone levels, higher PTH, insulin, HOMA-IR and triglycerides values; they also exhibited a lower functional independence degree and were engaged in a significantly poorer weekly LTPA.

Relationship of 25(OH)D with testosterone levels

The levels of 25(OH)D were strongly correlated with free testosterone ($r = 0.60$, $P < 0.0001$; Figure 1). Other significant correlates were total testosterone ($r = 0.42$, $P = 0.002$; Figure 1), PTH ($r = -0.49$, $P = 0.0003$), functional independence degree ($r = 0.36$; $P = 0.01$) and weekly LTPA ($r = 0.41$, $P = 0.003$).

A biochemical androgen deficiency (total testosterone <300 ng/dL) was observed in 17 patients (34.7%); it

was exhibited by 16 of 36 patients with 25(OH)D deficiency (44.4%) but only by 1 of 13 patients (7.7%) with 25(OH)D levels ≥ 20 ng/mL ($p = 0.02$).

The association of 25(OH)D with total and free testosterone was further explored with linear regression models (Table 2). Lower levels of 25(OH)D were associated with both lower total and free testosterone levels in unadjusted models. These associations persisted following adjustment for age, smoking, alcohol consumption, coexisting illness and HOMA-IR. However, after full adjustment, also including functional independence degree, BMI and weekly LTPA, only the independent association of lower 25(OH)D with lower free testosterone was still significant. Although total and free

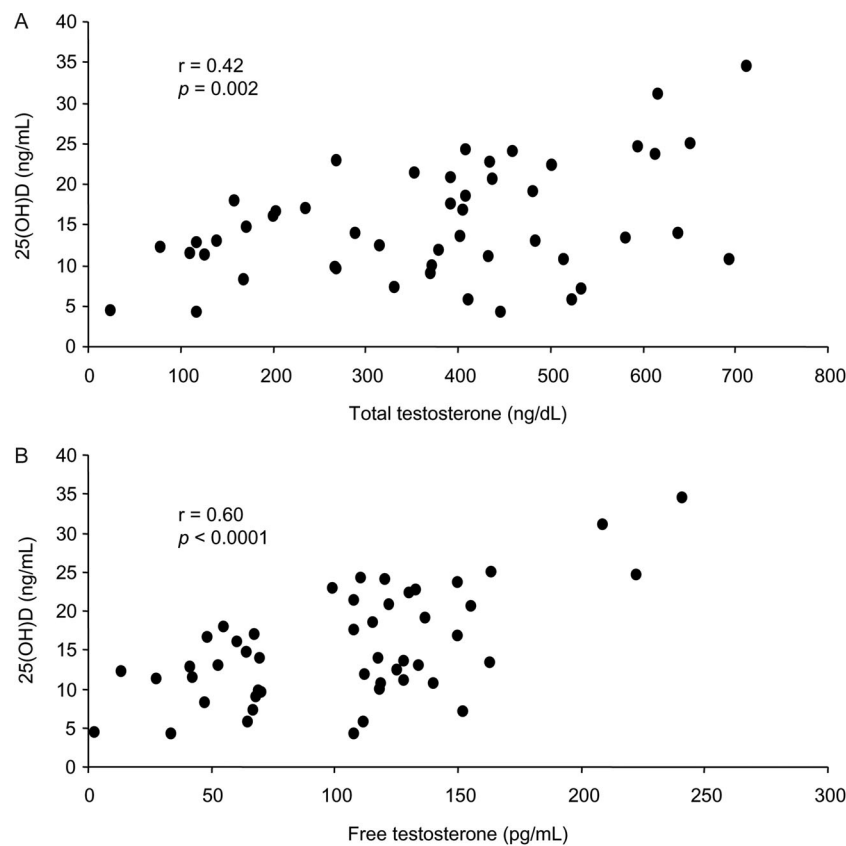


Figure 1 Correlations of 25(OH)D with total testosterone (A) and free testosterone (B). To convert the values for 25(OH)D to nmol/L, multiply by 2.5; to convert the values for total testosterone to nmol/L, multiply by 0.03467; to convert the values for free testosterone to pmol/L, multiply by 3.467.

Table 2 Association between 25-hydroxyvitamin D and testosterone levels: linear regressions

	Model I	Model II	Model III
Total testosterone	0.30 (0.09, 0.50) P = 0.005	0.28 (0.02, 0.53) P = 0.03	0.29 (−0.04, 0.62) P = 0.08
Free testosterone	0.35 (0.18, 0.52) P = 0.0001	0.37 (0.17, 0.58) P = 0.0007	0.42 (0.17, 0.67) P = 0.001

Values are β -coefficients (95% CI). Model I, unadjusted; model II, adjusted for age, smoking, alcohol consumption, coexisting illness, and homeostatic model assessment of insulin resistance; model III, adjusted for model II+functional independence degree (Barthel index), body mass index and weekly leisure time physical activity.

testosterone were significantly correlated with HOMA-IR, BMI and weekly LTPA (not shown), a multicollinearity effect was ruled out, because the VIFs for independent variables in the regression analyses were lower than 2.6.

Discussion

While an association between 25(OH)D and testosterone levels has been reported in the general population,^{10–12} their relationship had not been investigated in men with chronic SCI, until a recent report published while this manuscript was in preparation.²² In that study a correlation between 25(OH)D and calculated free

testosterone levels was found in 17 controls, but not in 29 men with chronic SCI.

On the contrary, in the present study, 25(OH)D levels were correlated with total and free testosterone levels in men with SCI. In those with 25(OH)D deficiency (73.5%), a biochemical androgen deficiency was ~6 fold more prevalent than in those with 25(OH)D \geq 20 ng/mL (44.4% vs 7.7%). This would be expected, as vitamin D and androgen deficiency share some risk factors, including physical inactivity, overweight and coexisting illness, which are highly prevalent in this population. Nevertheless, the significant linear association between 25(OH)D and free testosterone levels

persisted after full adjustment for confounders. Actually, although residual confounders cannot be ruled out with certainty, this study indicates an association between 25(OH)D and free testosterone independent of major determinants of 25(OH)D/testosterone, including BMI and physical activity (model III, in Table 2). These data conflict with those from the above mentioned recent report where no correlation was found between 25(OH)D and total and free testosterone.²² Although discrepancy could be explained by differences in the size and characteristics of the study population, extremely low reference values for calculated free testosterone were indicated in that study, so that all values reported both in men with SCI and in control group were in the range of overt hypogonadism according to shared cut-off.^{30,33,34}

An independent linear association between 25(OH)D and testosterone had been previously reported in able-bodied men by two studies,^{10,11} but not by the EMAS, where it was lost after multivariate adjustment for health and lifestyle factors.¹² The evidence for an independent association between 25(OH)D and testosterone is in keeping with experimental/basic findings. Both 25(OH)D and testosterone deficiency might reflect a Leydig cell dysfunction. Leydig cells express CYP2R1 gene, encoding the main enzyme involved in 25-hydroxylation of vitamin D.³⁵ Furthermore, bilateral orchidectomy produced a significant decrease in 25(OH)D levels, not reverted by androgen replacement therapy.³⁶ On the other hand, some evidence also suggests a role for vitamin D in testosterone biosynthesis. VDR-knockout mice developed hypergonadotropic hypogonadism.³⁷ Furthermore, *in vitro* exposure of human Leydig cell cultures to 1,25(OH)D significantly increased testosterone synthesis, by modifying the expression profiles of steroidogenesis genes.³⁸ Obviously, due to its transversal design, the present study does not help to clarify the nature of this association: low vitamin D as a reflection of testicular dysfunction or playing a causal role in androgen deficiency.

Regardless of the independent link between vitamin D and free testosterone, the present study reinforces the notion that low vitamin D and androgen deficiency are markers of poor health, sharing common risk factors. In the regression analysis, when BMI and physical activity were included, the association of vitamin D with total testosterone was lost and that with free testosterone was attenuated. Both BMI and physical activity, measured as LTPA in men with chronic SCI, are life style-related modifiable risk factors. This could be relevant to clinical practice: in the rehabilitative approach to patients with SCI, a great effort should be made in

inducing modifications of these life style-related risk factors, shared by both hypovitaminosis D and androgen deficiency.

Our study suffers from some limitations. Firstly, the limited sample numerosness, which, however, was enough to demonstrate significant and independent associations, likely due to the high prevalence of both vitamin D and androgen deficiency in this populations. Another limitation is that total testosterone was measured by an immunoassay, which has lower accuracy than mass spectrometry, which, however, is rarely used in clinical settings.

Conclusion

An evidence is here provided that, in men with SCI, 25(OH)D correlates with total testosterone and free testosterone and exhibits an independent linear association with free testosterone. Regardless of this independent link, hypovitaminosis D and androgen deficiency share life style-related risk factors to take into account in the rehabilitative approach to patients with SCI.

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Disclaimer statements

Contributors AB designed the research, conducted the study, analyzed data and interpreted results, performed statistical analysis and wrote the manuscript; MRCV analyzed data; GF and SF revised the article critically and approved its final version; FF designed the research, analyzed data and interpreted results, wrote the manuscript, revised the article critically and approved its final version.

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Conflicts of interest The authors declare no potential conflict-of-interest.

Ethics approval The study was approved by the ethics committee of ASL1 Avezzano-Sulmona-L'Aquila.

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